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A Hand-Held Evanescent Wave Multianalyte Biosensor with Fully Disposable Components

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Elric W. Saaski

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Research International, Inc.
18706 142nd Ave. N.E.
Woodinville, WA 98072

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Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5660

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14. ABSTRACT

Phase I's primary objective was a handheld, multi-analyte, wet-chemistry instrument design that could measure a wide range of chemicals, proteins, viruses, bacteria and biological fragments at concentrations ranging to below one part per billion. This was to be accomplished through an integration of state-of-the-art digital and analog circuitry with novel miniaturized fluid handling technologies, optics, and highly specific antibody-based assay techniques. Successful implementation of a dot-style immunoassay strategy was demonstrated on a prototype molded polystyrene optical element. Experimental tests strongly substantiated the design strategy. A 5-channel electro-optic module was constructed that produced signal levels well within the measurement ability of state-of-the-art PIN photodiode technology, and experimental binding curves for model assays were remarkably smooth, lacking in noise, and exhibited no optical cross-talk between channels.

15. SUBJECT TERMS

Waveguide, Evanescent wave, Fluoroimmunoassay, Biosensor, Hand-held, Microfluidic, Portable, Anti-body.

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Final Report

Contract #: N00014-99-M-0203

Principal Investigator: Elric W. Saaski

Institution: Research International, Inc.

Grant Title: A Hand-Held Evanescent Wave Multianalyte Biosensor with Fully Disposable Components

Award Period: May 1, 1999 through October 31, 1999

Objective: To determine the feasibility of creating an 8-channel, evanescent wave-based multianalyte biosensor in a hand-held format.

Approach: To generate a viable hand-held instrument design, it was necessary to move beyond existing technology in optics and fluidics. Candidate optical designs for a disposable injection-molded assay coupon with integral waveguide were modeled and refined using the commercial design codes Zemax™ and Opticad™. Novel methods for pumping and routing fluids were examined, with a focus on reliability, small size and low power consumption. Upon development of baseline fluidic and optical designs we selectively built prototype hardware to demonstrate the feasibility of high risk and/or critical design features.

Accomplishments: Over the course of the program we doubled the design goal analyte capacity from 8 to 16. This was considered more in line with military and civilian first responder needs and not appreciably more risky to implement. The design is centered around a linear array of antibody-based assay 'dots' printed onto the surface of an optical waveguide. The dots are evanescently excited by a shared laser source, but each analyte assay 'dot' can be individually addressed with sample and assay reagents through a fluidic manifold and ganged valves that are integral to the coupon, that is, that derive from features built into the injection mold.

The optical design also incorporates novel aspheric molded optics for injecting light into the waveguide that minimize problems associated with laser-to-waveguide misalignment- these optical components also derive from surface features in the master mold and hence are very inexpensive, yet high in value. The method also provides an extremely high evanescent field strength for excitation of reporter fluorophores bound at each assay dot position.

Fluorophore-tagged antibody reagents for performing sandwich immunoassays are stored onboard the disposable assay coupon. Each detector spot has its own reagent reservoir and reagents are not mixed, eliminating cross-reaction concerns often raised when multiple-antibody reagent cocktails are used. Reagents are not rapidly exhausted by use and it is expected that each coupon and its reagents may be reused up to 30 times if no target species are present in analyzed samples, greatly minimizing consumables costs. With the assay card's high level

of functional integration, an unsophisticated user can run an assay by simply mounting a coupon in the instrument's docking bay and pressing a button.

Analytical modeling was performed and a novel multichannel pumping mechanism designed and prototyped that demonstrated the feasibility of pumping reagents in and out of comparatively small (10 to 100 μl) reservoirs with minimum energy expenditure. Power consumption was only 150 mW during the pumping process.

A prototype injection mold was also constructed for the most promising coupon design and polystyrene sample parts obtained from the mold were used in proof-of-principle assays. Excitation light for these tests was provided by a 635 nm laser diode (330 μW of optical power into the waveguide), while fluorescence signals were collected by a 5-channel receiver cradle that used GRIN lenses in combination with a custom sharp-cut dichroic filter to reject stray excitation light.

In the optically excited zone of the molded article, capture antibody dots of 0.25 to 1.0 mm diameter were printed and when the coupon was mounted in the receiver cradle, the dots were positioned at the respective receiver channel focal points. For proof-of-principle tests a model assay was used where the 'analyte' was Cy5-labeled goat IgG, while rabbit anti-goat IgG was used as the surface-attached capture antibody.

Standard PIN photodiodes were used in conjunction with high quality analog amplifiers having a current resolution of 0.025 pA. It was found that typically, photocurrents of 100-200 pA were obtained under saturated capture site conditions for 1 mm diameter spots. On the low end, minimum concentrations clearly resolved after an incubation time of five minutes were in the range of 1-10 ng/ml of Cy5-labeled goat IgG.

Conclusions: Evanescent-wave excited immunoassays were successfully performed with 1mm diameter monolayer-coated assay dots on a molded polystyrene waveguide structure. A sensitivity of 1-10 ng/ml of target analyte was achieved using a conventional uncooled PIN photodiode technology with only 330 μW of optical excitation.

Significance: Enabling technologies for a 16-channel hand-held bioassay instrument have been demonstrated. The approach has adequate sensitivity and multianalyte capability to meet the needs of diverse applications such as biowarfare agent detection, environmental monitoring, and food safety, and uses a disposable assay card that may be used up to 30 times before discarding. Demonstration of application-relevant analyte sensitivity is particularly encouraging since it was done at a low excitation power level and without the need for photomultipliers or cooled CCD arrays and the like. This augers for a compact, low power device that should be easy to manufacture, rugged, and within the budgetary limits of a broad spectrum of users.

Patent Information: No patent has been filed.

Award Information: None

Publications and Abstracts: None